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# Short communication

# Floral Scent Chemistry and Stamen Movement of *Chimonanthus praecox* (L.) Link (Calycanthaceae)

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The floral scent of *Chimonanthus praecox* (L.) Link (Calycanthaceae; Magnoliids) was collected by the headspace method and analyzed using gas chromatography - mass spectrometry (GC-MS). The main components of the floral scents are benzyl acetate (42.2 %), *trans*-β-ocimene (24.8 %), linalool (17.2 %), and benzyl alcohol (6.9 %). These compounds are common in the scent profiles of many plants throughout the angiosperms. In addition, the female and male stages (phases) of the protogynous, drooping flowers of *C. praecox* f. *concolor* Makino were observed. In the female phase the stamens are bent toward the tepals away from the pistils at a right angle. After approximately two days the stamens commence to move to enclose the pistils. It takes from one to four days for the stamens to eventually enclose the pistils in the various flowers and then anthers shed their pollen.

Key words: fragrance, floral biology, protogynous, volatiles, Magnoliids

Floral scent is an important insect attractant and undoubtedly played a key role in the evolution of reproductive systems of flowering plants (Pellmyr & Thien 1986). However, the floral scent chemistries of most native and cultivated plants have not been analyzed (Knudsen *et al.* 1993).

This study analyzed the floral scent emitted from the yellow and nectarless flowers of *Chimonanthus praecox* (L.) Link (Calycanthaceae), native to southeastern China and widely cultivated in gardens. The plants of *C. praecox*, commonly known as winter sweet, flower December to February in Japan. In addition, we recorded the conspicuous movements of the stamens in relation to the functional life of the flower. Floral movements are common in other protogynous Magnoliids (Endress 1990, Thien *et al.* 2000).

## Materials and Methods

Samples of floral scent were collected from five cultivated plants (three individuals of *Chimonanthus praecox* and two of *C. praecox* f. *concolor* Makino) and analyzed using gas chromatography - mass spectrometry (GC-MS) from January 14 to 23, 2001. Twigs (n=5) with attached flowers (17-28 flowers / individual) were placed in water and enclosed in a polypropylene bag. The air in the bag was sampled using a mini-pump with a flow rate of 150ml/min via a cartridge containing 50 mg of adsorbent, Tenax TA 60/80 mesh, (inserted in the line) for four hours within 11:00-17:00. The scent volatiles trapped on the adsorbent were eluted with 300 μl diethyl ether. A 10 μl aliquot of nonyl acetate solution (0.5 mg/ml dichloromethane) was added to

all samples as an internal standard to calculate amounts of the volatiles. Two μl of the eluent was used for GC-MS analysis. Controls (polypropylene bag and twigs with no flowers) were simultaneously collected for all samples. Further details of floral scent sampling and analysis are described in Azuma *et al.* (2001, 2002, 2004). Observations of stamen movement were conducted on one plant of *C. praecox* f. *concolor* cultivated in the Kyoto University Botanical Garden from January 25 to February 2, 2001.

## Results and Discussion

Chemical profiles of the five floral scent samples collected from *Chimonanthus praecox* (including f. *concolor*) are presented in Table 1. The main components of the floral scent are benzyl acetate (42.2 %), *trans*-β-ocimene (24.8 %), linalool (17.2 %), and benzyl alcohol (6.9 %). These compounds are common in the scent profiles of many plants throughout the angiosperms (Knudsen *et al.* 1993). For example, benzyl acetate is the main component in the floral scent of *Sansevieria cylindrica* (Dracaenaceae) (Knudsen & Tollsten 1993), *Silene nutans*, *S. dichotoma*, *S. italica* (Caryophyllaceae) (Knudsen & Tollsten 1993, Jurgens *et al.* 2002), *Hesperis matronalis* (Cruciferae) (Nielsen *et al.* 1995), *Clarkia breweri* (Onagraceae) (Raguso & Pichersky 1995), *Narcissus papyraceus*, *N. serotinus* (Amaryllidaceae) (Dobson *et al.* 1997), and several orchid species (Williams & Whitten 1983, Gerlach & Schill 1991). In addition, benzyl acetate has been used to trap various species of beetles in the fields (Ikeda *et al.* 1993, Nakashima *et al.* 1994, Fukuyama 1995). The floral scent profile of *C. praecox* is

TABLE 1. Chemical profiles of floral scents emitted from *Chimonanthus praecox* (including f. *concolor*). Compounds marked with asterisk (*) were identified by mass spectra and GC-retention times of authentic compounds.

| | *Chimonanthus praecox* | | | f. *concolor* | | Average |
|---|---|---|---|---|---|---|
| No. of flower / sample | 27 | 28 | 17 | 18 | 19 | |
| Total μg / fl / 4hrs | 3.21 | 1.16 | 2.21 | 2.09 | 6.33 | 3.00 |
| ***Benzenoids*** | | | | | | |
| Benzealdehyde*[1)] | 0.6 | 2.3 | 3.3 | 2.5 | tr[2)] | 1.7 |
| Benzyl alcohol* | 1.6 | 7.4 | 11.3 | 9.9 | 4.4 | 6.9 |
| Methyl benzoate*[1)] | 0.2 | 1.3 | 0.9 | 0.9 | 1.2 | 0.9 |
| Benzyl acetate* | 41.5 | 59.0 | 42.3 | 26.8 | 41.6 | 42.2 |
| Cinnamyl acetate* | 0.2 | -[3)] | - | - | - | <0.1 |
| ***Terpenoids*** | | | | | | |
| Limonene*[1)] | 0.3 | 1.0 | 1.1 | 0.5 | 7.4 | 2.1 |
| *cis*-β-Ocimene* | 0.9 | - | tr | 0.3 | - | 0.3 |
| *trans*-β-Ocimene* | 39.2 | - | 21.9 | 43.5 | 19.3 | 24.8 |
| Linalool* | 13.8 | 20.9 | 17.9 | 14.2 | 19.4 | 17.2 |
| 2,6-dimethyl-2,4,6-octatriene | 0.2 | - | - | - | - | <0.1 |
| ***Fatty acid derivatives*** | | | | | | |
| Undecane*[1)] | tr | 0.5 | 0.5 | 0.0 | 4.7 | 1.2 |
| Dodecane*[1)] | 0.2 | 0.8 | 0.7 | 0.7 | 2.0 | 0.9 |
| 43 67 71 95 113 | tr | 4.4 | tr | 0.7 | - | 1.0 |
| 43 67 71 95 113 | tr | 2.5 | tr | tr | tr | 0.5 |
| ***N-compound*** | | | | | | |
| Indole* | 1.3 | tr | - | - | - | 0.3 |
| Total (%) | 100 | 100 | 100 | 100 | 100 | 100 |

[1)] These compounds were also found in the controls (bag and twigs with no flowers) with nearly equal amount.
[2)] trace amount.
[3)] not detected.

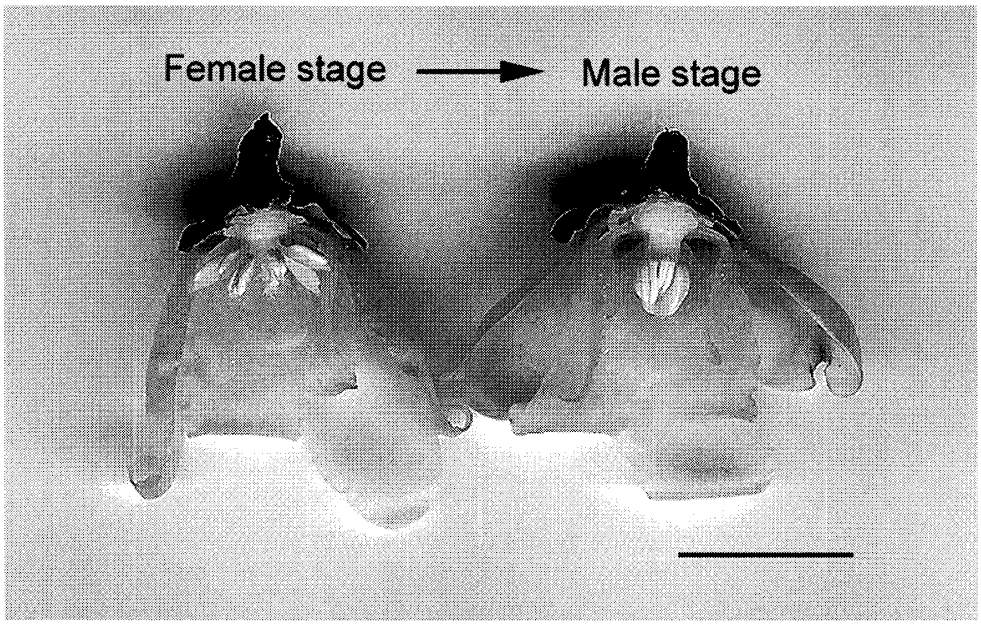


FIG. 1. Female and male stage (phase) flowers of *Chimonanthus praecox* f. *concolor*. Stamens of the female stage (phase) flower are showing early movement of stamens. Bar indicates 1 cm.

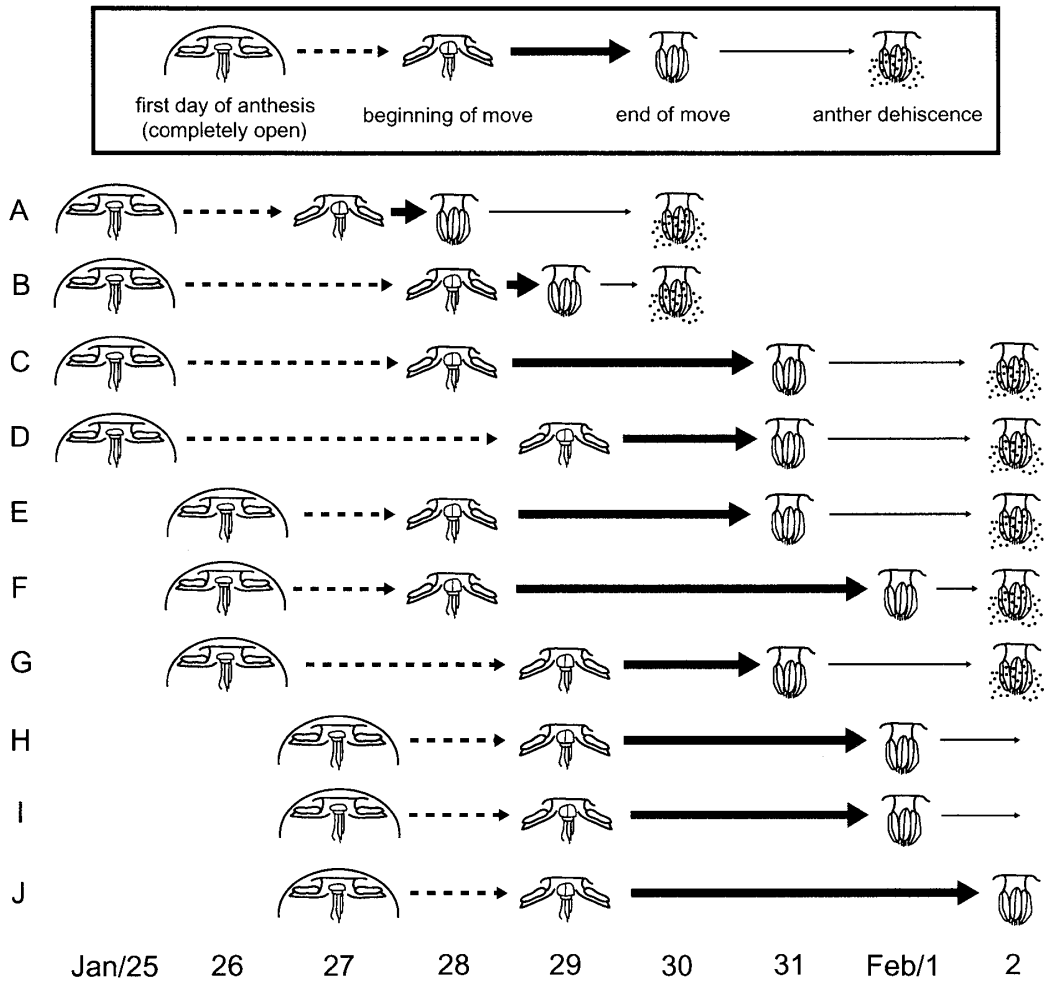


FIG. 2. Stamen movement of *Chimonanthus praecox* f. *concolor*. Ten flowers (A-J) were observed.

representative of many insect pollinated species of plants. Although the pollination biology of *C. praecox* is not well studied, Liu *et al.* (1999) noted that the flowers were pollinated by flies and honeybees.

There is no apparent difference between chemical profiles of *Chimonanthus praecox* and *C. praecox* f. *concolor* in quality and quantity of chemicals (Table 1). However, one of three samples of the floral scent of *C. praecox* lacked *trans*-β-ocimene and showed a large amount of benzyl acetate (59.0 %) and other compounds (Table 1). Intraspecific variation in floral scent chemistry is often observed (*e.g.,* Tollsten & Bergstrom 1993, Azuma *et al.* 2001) and additional samples from natural populations need to be analyzed to display the degree of variation of floral scent profiles in *C. praecox*.

The female and male stages (phases) of the protogynous, drooping flowers of *Chimonanthus praecox* f. *concolor* are shown in Fig. 1. We observed the movement of the stamens and throughout the flowering of ten flowers (one plant; Figs. 1 & 2). In the female phase the stamens are bent toward the tepals away from the pistils at a right angle (Figs. 1 & 2). After approximately two days the stamens commence to move to enclose the pistils (Fig. 2). It takes from one to four days for the stamens to eventually enclose the pistils in the various flowers and then anthers shed their pollen (Fig. 2). The flowers need to be self and cross- pollinated by hand to determine if the plants can be inbreed and also to determine the functional period of the female stage. Preliminary observations suggest the flowers are adapted for out-crossing.

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## References

Azuma, H., M. Toyota & Y. Asakawa. 2001. Intraspecific variation of floral scent chemistry in *Magnolia kobus* DC. (Magnoliaceae). J. Plant Res. 114: 411-422.

—, —, —, T. Takaso & H. Tobe. 2002. Floral scent chemistry of mangrove plants. J. Plant Res. 115: 47-53.

—, V. Rico-Gray, J. G. Garcia-Franco, M. Toyota, Y. Asakawa & L. B. Thien. 2004. Close relationship between Mexican and Chinese *Magnolia* (subtropical disjunct of Magnoliaceae) inferred from molecular and floral scent analyses. Acta Phytotax. Geobot. 55: 167-180.

Dobson, H. E. M., J. Arroya, G. Bergström & I. Groth. 1997. Interspecific variation in floral fragrances within the genus *Narcissus* (Amaryllidaceae). Biochem. Syst. Ecol. 25: 685-706.

Endress, P. K. 1990. Evolution of reproductive structures and functions in primitive angiosperms (Magnoliidae). Mem. New York Bot. Gard. 55: 5-34.

Fukuyama, K. 1995. How to study the canopy beetles in a tropical rain forest. - research trial in the Pasoh Forest Reserve, Peninsular Malaysia. Tropics 4: 317-326. (in Japanese with English summary)

Gerlach, G & R. Schill. 1991. Composition of orchid scents attracting euglossine bees. Bot. Acta. 104: 379-391.

Ikeda, T., E. Ohya, H. Makihara, T. Nakashima, A. Saitoh, K. Take & K. Kojima. 1993. Olfactory responses of *Anaglyptus subfasciatus* Pic and *Demonax transilis* Bates (Coleoptera: Cerambycidae) to flower scents. J. Jpn. For. Soc. 75: 108-112.

Jurgens, A., T. Witt & G. Gottsberger. 2002. Flower scent composition in night-flowering *Silene* species (Caryophyllaceae). Biochem. Syst. Ecol. 30: 383-397.

Knudsen, J. T. & L. Tollsten. 1993. Trends in floral scent chemistry in pollination syndromes: floral scent composition in moth-pollinated taxa. Bot. J. Linn. Soc. 113: 263-284.

—, — & L. G. Bergström. 1993. Floral scents - a checklist of volatile compounds isolated by headspace techniques. Phytochemistry 33: 253-280.

Liu, H., Y. Xu & F. Yang. 1999. The flowering season and pollination agent of members belonging to Caly-

canthaceae. J. Beijing For. Univ. 21: 121-123. (in Chinese with English summary)

Nakashima, T., K. Nakamuta, H. Makihara, E. Ohya, M. Nakanishi & T. Ikeda. 1994. Field response of *Anaglyptus subfasciatus* Pic (Coleoptera: Cerambycidae) to benzyl acetate and structurally related esters. Appl. Entomol. Zool. 29: 421-425.

Nielsen, J. K., H. B. Jakobsen, P. Friis, K. Hansen, J. Moller & C. E. Olsen. 1995. Asynchronous rhythms in the emission of volatiles from *Hesperis matronalis* flowers. Phytochemistry 38. 847-851.

Pellmyr, O. & L. B. Thien. 1986. Insect reproduction and floral fragrances: keys to the evolution of the angiosperms? Taxon 35: 76-85.

Raguso, R. & E. Pichersky. 1995. Floral volatiles from *Clarkia breweri* and *C. concinna* (Onagraceae): recent evolution of floral scent and moth pollination. Plant Syst. Evol. 194: 55-67.

Thien, L. B., H. Azuma & S. Kawano. 2000. New perspective on the pollination biology of basal angiosperms. Int. J. Plant Sci. 161 (6 Suppl.): S225-S235.

Tollsten, L. & L. G. Bergström. 1993. Fragrance chemotypes of *Platanthera* (Orchidaceae) - the result of adaptation to pollinating moths? Nord. J. Bot. 13: 607-613.

Williams, N. H. & W. M. Whitten. 1983. Orchid floral fragrances and male euglossine bees: methods and advances in the last sesquidecade. Biol. Bull. 164: 355-395.

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